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A quantitative trait locus for reduced culm internode length in barley segregates as a Mendelian gene

M. Sameri · S. Nakamura · S. K. Nair · K. Takeda · Takao Komatsuda

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Abstract Yield losses caused by lodging in cereals can be partially controlled by reducing plant height. A progeny of recombinant inbred lines from a cross of two Japanese barley varieties was used to study the inheritance of culm and culm internode lengths. An unexpected QTL for reduced culm length (*qCUL*), which affected mainly the length of the third and fourth culm internodes, was contributed by 'Kanto Nakate Gold'. This QTL was also associated with reduced lodging in two experiments. A near-isogenic line (culm length 62.9–73.4 cm) in an 'Azumamugi' background, carrying a chromosome segment containing the *qCUL* allele from Kanto Nakate Gold, was significantly shorter than its recurrent parent (82.9–89.4 cm). The F_2 generation from the next backcross segregated for plant height in a Mendelian monogenic ratio. The *qCUL* locus was shown to be tightly linked (1.2 cM) with the codominant STS marker *ABG608*.

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M. Sameri · S. K. Nair · T. Komatsuda (✉)
National Institute of Agrobiological Sciences (NIAS),
Tsukuba, Ibaraki 305-8602, Japan
e-mail: takao@affrc.go.jp

S. Nakamura
National Institute of Crop Science (NICS),
Tsukuba, Ibaraki 305-8518, Japan

K. Takeda
Research Institute for Bioresources, Okayama University,
Kurashiki, Okayama 710-0046, Japan

Introduction

Lodging, the permanent displacement of stems from the vertical, is a problem in most cereals and in some situations can reduce the grain yield of barley (*Hordeum vulgare*) by as much as 65% (Jedel and Helm 1991). When a cereal crop suffers extensive lodging, fungal disease development on the leaves and spikes is encouraged, and the efficiency of mechanical harvesting is drastically reduced (Tar'an et al. 2003). The tendency to lodge is a function of stem strength, so short plant stature minimizes the risk of lodging as well as increasing the harvest index (Bezant et al. 1996; Hellewell et al. 2000). The introduction of semi-dwarfing genes was the single most important contributor to the improvement of grain yield in 'Green Revolution' wheat and rice (Ishimaru et al. 2004), and a similar change in plant height has been achieved by the deployment of several dwarfing genes in barley. Thus, most European barley cultivars carry the denso (semidwarf 1, *sdw1.c*) gene (Bezant et al. 1996; Kicherer et al. 2000), while the stature of many Japanese and Korean short cultivars is conditioned by the uzu 1 (*uzu1*) gene (Takahashi and Yamamoto 1951; Franckowiak and Konishi 1997a), which also imposes pleiotropic effects on the length of the spike, awn and culm (Sameri et al. 2006). Another major gene in common use in Japan and Korea (Takahashi 1951; Franckowiak and Konishi 1997b), and one which affects these same characters is dense spike 1 (*dsp1*) (Sameri et al. 2006). Ear type (two-row vs. six-row) in barley is determined by six-rowed spike 1 (*vrs1*), which is also pleiotropic for several morphological characters, including the number of rachis nodes, the length of the spike and stem and the heading date (Marquez-Cedillo et al. 2001; Sameri and Komatsuda 2007).

In addition to the semi-dwarfing and dwarfing genes, a number of quantitative trait loci (QTL) are also known to

affect plant height in barley. Such QTL have been identified on all seven barley chromosomes (Hayes et al. 1993; Pillen et al. 2003), and some of these may have breeding value to increase lodging resistance. Their identification has relied largely on the measurement of final plant height, but their effect on the length of each internode has not been analyzed. The lower internodes (particularly the second to the sixth) are of particular relevance to the lodging characteristics of a genotype (Berry et al. 2006). Sameri et al. (2006) reported that several QTLs controlled culm length. *qCUL* was novel among these QTL, located on chromosome 7HL. The objective of the present study was to elucidate the pattern of inheritance of not only culm length but also length of each culm internode in barley. For this purpose, we selected desirable backcrossed lines (BC_4F_7) characterized by whole genome survey using STS and SSR markers, in which only *qCUL* would be segregated as a single genetic factor.

Materials and methods

Field trials

A population of 99 F_{13} recombinant inbred lines (RILs,) was developed by the single seed descent method from the cross 'Azumamugi' × 'Kanto Nakate Gold'. Azumamugi (AZ) and Kanto Nakate Gold (KNG) are held in the Okayama collection as OUJ 698 and OUJ518 and their description is in the Catalogue of Barley Germplasm preserved in Okayama University (Takahashi et al. 1983) or the website http://www.rib.okayama-u.ac.jp/barley. The two cultivars differ from one another with respect to a number of morphological and physiological traits (Komatsuda et al. 1993, 2004). AZ has a six-rowed spike 1 (*vrs1*), uzu 1 (*uzu1*) gene or semi-brachytic growth with 10–15 cm shorter than KNG, dense spike 1 (*dsp1*), and a winter type growth (*Sgh1/sgh2*). KNG has an opposite genotype for these genes. The materials were field sown on 28 October 2004 and 18 October 2006 at Tsukuba (36° 2′ 8″ N, 140° 4′ 26″ E) and harvested in the summer of 2005 and 2007, respectively (hereafter referred as '2005' and '2007'). A set of 141 BC_4F_7 NILs was developed from the cross AZ (recurrent parent) × KNG (donor parent) (T. Komatsuda, unpublished data). This material was field sown at Tsukuba on 21 October 2003 and 13 October 2005 and harvested in the summer of 2004 and 2006 (hereafter referred as '2004' and '2006', respectively). NIL#11, was crossed with AZ, and 86 F_2 plants were grown in the field of summer 2006. Fertilizers were applied at the rate of N–P_2O_5–K_2O = 18–27–18 kg/ha to the soil prior to cultivation. Field management was in accordance with the local practice and there was no irrigation.

Experiments on RILs were performed for 2 years (2005 and 2007), and experimental design for each year consisted of a complete block design with one replication. Each RIL and the two parental lines were represented by one row of 10 plants with 20 cm between plants and 80 cm between rows. Four plants were selected from the center of each row, and the four main tillers of each plant were scored for culm length and spike length at maturity. The culm was partitioned into its internodes, with the first internode (IN1) representing the portion from the collar (base of spike) to the uppermost node (so that IN1 is identical to 'peduncle length' as described in Sameri et al. 2006). IN2–IN6 were counted downwards from the uppermost node. Ear (or rachis) internode length (SIL), spike length (SPL), triplet number (TPN) and culm length (CUL) were measured in the same way as reported previously (Sameri et al. 2006). In our previous trials of 2001 and 2002 for agronomic traits (Sameri et al. 2006), lodging (LDG) of RILs was calculated as the % of the row which had suffered lodging × angle to the vertical. Therefore, a crop lying horizontally has an angle of 90° (CIMMYT 1998). The culm length of four central plants (2004) and eight plants (2006) was measured from each NIL at maturity.

Statistical analysis

The measured values of the four plants per RIL were averaged, and analysis of variance of the randomized complete block design (a year as block) has been performed. The broad sense heritability (h^2b) was calculated from the ANOVA table as $h^2b = \sigma_G^2/(\sigma_G^2 + \sigma_G^2/r)$, where $\sigma_G^2 = (\mathrm{MS}_{\mathrm{genotype}} - \mathrm{MS}_{\mathrm{Error}})/r$, $\sigma_E^2 = \mathrm{MS}_{\mathrm{Error}}$, and r = number of replications. The lodging data were arcsine transformed for normalization (Thomas and Jackson Hills 1978). Significant mean differences between each NIL and its recurrent parent for culm length were calculated by means of a *t* test.

QTL mapping in the RIL and NIL population

Of the 134 SSR (simple sequence repeat) markers (Ramsay et al. 2000), the 42 which were polymorphic between AZ and KNG (S. Nakamura, unpublished data) were used to genotype the 99 RILs, and their map locations were integrated into an earlier version of this map (Mano and Komatsuda 2002). PCR amplification procedure of SSRs marker was performed by an initial denaturation step of 5 min at 94°C followed by 30 cycles of three steps: denaturation for 30 s at 94°C, annealing for 30 s at 55–60°C, extension for 30 s at 72°C with a final extension for 7 min at 72°C.

The linkage map was constructed using the RI model in Mapmaker/EXP v3.0 (Lander and Botstein 1989). For QTL

analysis, composite interval mapping (CIM) was applied using Windows QTL Cartographer v2.0 (Wang et al. 2004). LOD thresholds for the presence of QTL in each trait were estimated by a 1,000 permutation test. Among the NIL materials, 68 markers were applied, consisting of 21 SSRs, 47 sequence tagged sites (STS), three morphological traits six-rowed spike (*vrs1*), cleistogamy (*cly1*), and uzu or semi-brachytic (*uzu1*). STS analysis followed established methods (Sayed-Tabatabaei et al. 1999; Mano et al. 1999). A χ^2 test was applied to test observed segregation ratios among the BC_5F_2 progenies (NIL#11 × AZ) for culm and internode length.

Results

Genetic linkage map

The 42 informative SSR markers mapped across all seven barley chromosomes, to give a total map length of 995.7 cM (Fig. 1a, ESM1). The order of the loci was in good agreement with that of Ramsay et al. (2000) and Li et al. (2005), except for *Bmag0222*, which mapped to chromosome arm 5HS rather than 5HL. This SSR assay generated an amplification profile consisting of a 175 and 150 bp fragment, and the former (dominant for AZ) was scored in the RIL population.

Culm internode length

Plant height consists of culm length and spike length. Both culm length and plant height were controlled by five common QTLs (Sameri et al. 2006), therefore we described only culm length in this study. The culm and culm internode lengths among the RIL population are given in Table 1. Transgressive segregation was present for each internode. The analysis of variance showed a significant genotypic effect for all the traits (Table 2). The allelic variation at the *uzu1* locus explained 39–51% of the total phenotypic variance present for IN1 (Table 3). A QTL for IN1, designated *qCUL*, explained 6.6% of the phenotypic variance, and mapped between *EBmag757* and *ABG608* on chromosome 7H. Finally, *vrs1* explained 5.6% of the variance. IN1 was increased by the AZ allele at *qCUL,* but decreased by the AZ alleles at *uzu1* and *vrs1*. *dsp1* had no significant effect on IN1, as reported previously (Sameri et al. 2006). IN2–IN4 were under the control of *uzu1, dsp1* and *qCUL* (Table 3). *qCUL* explained 28% (IN3) and 29% (IN4) of the variance in 2005, and 11%, 17% in 2007. IN4 was also affected by *sgh1* and *Eam5,* with the former explaining 11% of the variance. IN5 and IN6 were primarily controlled by *sgh1* and *Eam5* (Table 3), with a positive effect being exerted by the AZ allele at the former, but a negative one at the latter.

Spike characteristics

SIL and SPL were controlled by three loci (*qSIL.ak-2HL, uzu1* and *dsp1*) (Table 3). Allele of KNG at *qSIL.ak-2HL* locus on 2HL made spike internode length shorter than did allele of AZ (Sameri et al. 2006). TPN was controlled by five loci (*eam8, Ppd-H1, vrs1, Eam5* and an unnamed QTL) (Table 3). The TPN QTL located at the interval *ABC261–ABG055* on 1HL is closely linked or allelic to the *eam8* (early maturity 8) gene (Sameri and Komatsuda 2004; Sameri et al. 2006).

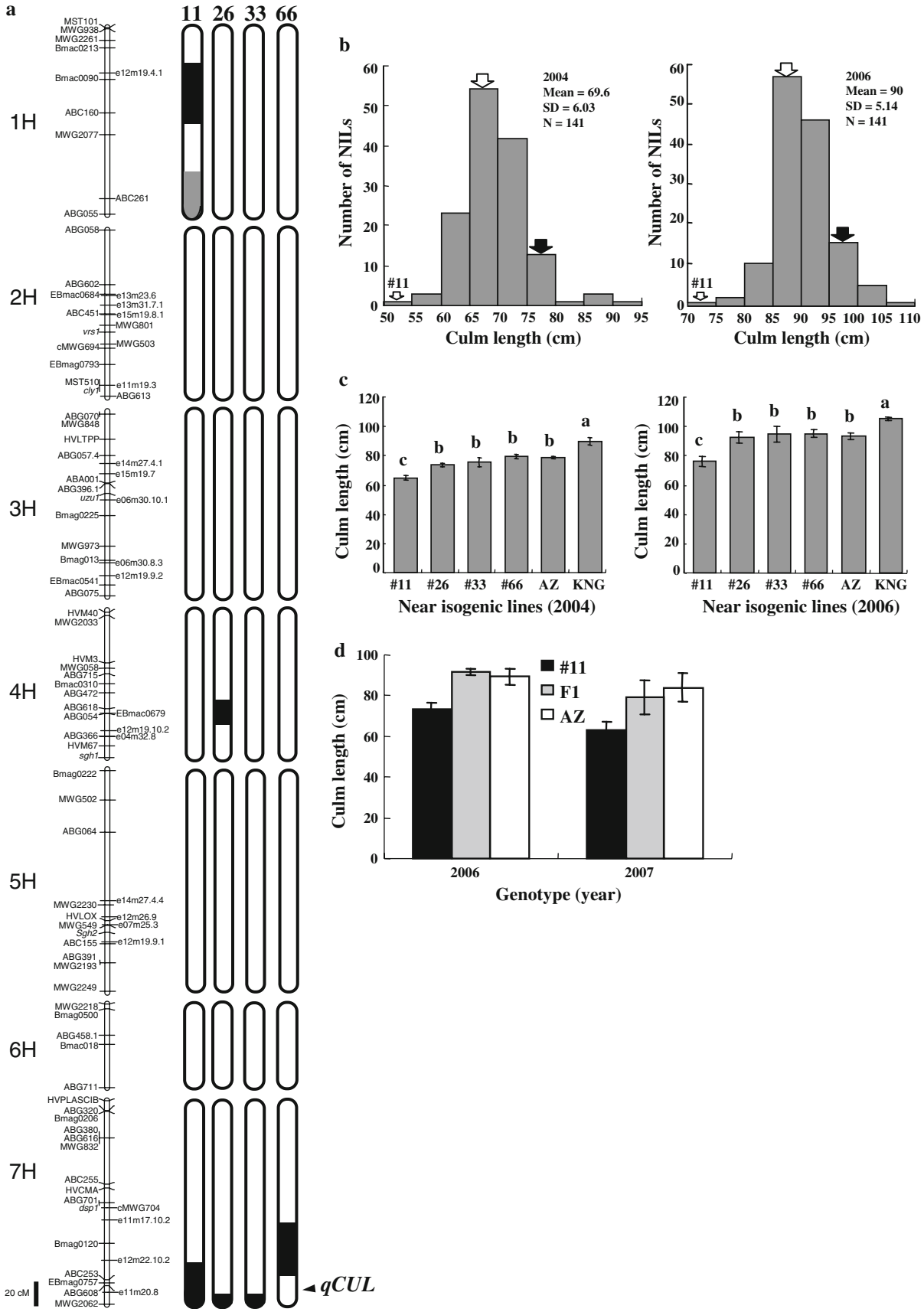
The main phenotypic effect of *vrs1*, which has recently been isolated and shown to encode a homeodomain protein (Komatsuda et al. 2007), is to determine the spike type as two- or six-row. Many studies (Takahashi et al. 1975; Kjaer et al. 1995; Marquez-Cedillo et al. 2001; Sameri et al. 2006) have shown that *vrs1* acts pleiotropically to reduce IN1, but not the length of the other internodes. This effect was reproduced in the present study.

Lodging

There was a significant effect of genotype on lodging (Table 2). *qCUL* influenced LDG significantly in both years (Table 3). The correlation between LDG and CUL was highly significant ($r = 0.407$, $P < 0.01$) (ESM2, ESM3). The correlations between the LDG and each of IN1–IN6 suggested that IN3 had the largest effect on lodging ($r = 0.414$, $P < 0.01$). The KNG allele decreased the length of the lower internodes, and therefore decreased LDG.

NIL-based mapping of *qCUL*

NIL#11 was considerably shorter than the other lines in both years (Fig. 1b, c). This line carries a maximum of 46.2 cM chromosome 7H segment distal to *Bmag0120* in chromosome 7HL, inherited from KNG (Fig. 1a). NILs #26, #33 and #66 also carry a KNG segment in chromosome 7HL (Fig. 1a), but these three NILs were not significantly different from AZ for culm length (Fig. 1c). Based on this result *qCUL* was delimited to a 19.3 cM region between *ABC253* and *MWG2062* (Fig. 1a). None of the NILs carried *sgh1* or *Sgh2* as confirmed by the test of growth habit (data not shown) and molecular markers (Fig. 1a). NIL#11 and #26 have also inherited a second segment from KNG, involving, respectively, parts of chromosomes 1H and 4H, but neither of these genomic regions includes a locus for plant height or culm length (Table 3). Therefore, the variation for internode length was


a
11 26 33 66
1H
2H
3H
4H
5H
6H
7H
MST101
MWG938
MWG2261
Bmac0213
e12m19.4.1
Bmac0090
ABC160
MWG2077
ABC261
ABG055
ABG058
ABG602
EBmac0684
e13m23.6
e13m31.7.1
ABC451
e15m19.8.1
MWG801
vrs1
cMWG694
MWG503
EBmag0793
MST510
e11m19.3
cly1
ABG613
ABG070
MWG848
HVLTPP
ABG057.4
e14m27.4.1
e15m19.7
ABA001
ABG396.1
uzu1
e06m30.10.1
Bmag0225
MWG973
Bmag013
e06m30.8.3
e12m19.9.2
EBmac0541
ABG075
HVM40
MWG2033
HVM3
MWG058
ABG715
Bmac0310
ABG472
ABG618
ABG054
EBmac0679
e12m19.10.2
ABG366
e04m32.8
HVM67
sgh1
Bmag0222
MWG502
ABG064
MWG2230
e14m27.4.4
HVLOX
e12m26.9
MWG549
e07m25.3
Sgh2
ABC155
e12m19.9.1
ABG391
MWG2193
MWG2249
MWG2218
Bmag0500
ABG458.1
Bmac018
ABG711
HVPLASCIB
ABG320
Bmag0206
ABG380
ABG616
MWG832
ABC255
HVCMA
ABG701
dsp1
cMWG704
e11m17.10.2
Bmag0120
e12m22.10.2
ABC253
EBmag0757
ABG608
e11m20.8
MWG2062
20 cM
qCUL
b
2004
Mean = 69.6
SD = 6.03
N = 141
Number of NILs
#11
Culm length (cm)
2006
Mean = 90
SD = 5.14
N = 141
Number of NILs
#11
Culm length (cm)
c
Culm length (cm)
#11 #26 #33 #66 AZ KNG
Near isogenic lines (2004)
Culm length (cm)
#11 #26 #33 #66 AZ KNG
Near isogenic lines (2006)
d
#11
F1
AZ
Culm length (cm)
2006
2007
Genotype (year)

◀ **Fig. 1** The effect of *qCUL* on the culm length. **a** Genetic map based on 99 RILs and graphical genotypes of four critical NILs. Genes and markers used to characterize NILs were shown on the *left side* of the map, and the other markers used to indicate the intervals for QTLs were shown on the *right side* of the map (See ESM1 for the complete map). *Open bars* represent AZ segments, *solid bars* represent KNG segments and *patterned bars* represent heterozygous segments. **b** Frequency distribution among 141 NILs for culm length in two seasons. *Open arrow* AZ, *solid arrow* KNG. **c** Culm length of four critical NILs in 2006. **d** Culm length of NIL#11, AZ and the F_1 NIL#11 × AZ

due to genes in the chromosome 7H segment, and probably to *qCUL*. As the CUL of AZ and the F_1 NIL#11 × AZ were not significantly different from one another (Fig. 1d), *qCUL* acts as a nearly completely dominant gene. The extreme shortness of NIL#11 is caused by the pyramiding of *qCUL* with *uzu1* and *vrs1*, both of which are present in the recurrent parent AZ (Fig. 1a). Each *qCUL-K* and *qCUL-A* gene pair showed a significantly different culm length in all the combinations of *uzu*/+ and *vrs1*/+in RILs subpopulation (ESM4 figure). In the F_2 there was a bimodal distribution for CUL and IN3 (Fig. 2a, b), with the former segregating 61 tall (79–97 cm) and 25 short (63–77 cm) for CUL, and 60 long (11.5–14.5 cm), 26 short (8–11 cm) for IN3. IN3 was highly correlated with CUL (Fig. 2c). These segregation ratios are consistent with the action of a completely dominant single gene (CUL $\chi^2 = 0.76$, $0.3 < P < 0.5$; IN3 $\chi^2 = 1.26$, $0.2 < P < 0.3$). *qCUL* was tightly linked (1.2 cM distal) to the codominant STS marker *ABG608* (bin 7H12) (Fig. 2d).

Table 1 Mean, standard deviation and coefficient of variance for the components of plant height in 'Azumamugi', 'Kanto Nakate Gold' and derived recombinant inbred lines (RILs)

Traits[a]	IN1 (cm)	IN2 (cm)	IN3 (cm)	IN4 (cm)	IN5 (cm)	IN6 (cm)	CUL (cm)	SIL (mm)	SPL (cm)	TPN	LDG[b]
Year 2005											
Azumamugi	35.9	18.5	12.9	9.5	5.8	0.3	82.9	2.0	5.3	27.4	0.0
Kanto Nakate Gold	47.3	18.5	15.0	11.0	4.4	0.8	97.0	2.3	7.3	33.4	0.0
RIL, minimum	20.6	12.0	6.1	4.4	1.2	0.0	49.2	1.3	3.6	22.9	0.0
RIL, maximum	53.5	26.6	18.7	14.9	10.8	6.0	111.5	4.0	12.0	38.8	90.0
RIL, mean	38.6	19.5	13.0	9.7	5.2	0.9	86.8	2.3	6.8	30.2	30.1
RIL, SD[c]	6.6	3.2	2.3	2.5	2.2	1.2	12.8	0.7	2.1	3.3	32.2
CV (%)[d]	17.1	16.3	17.9	25.3	42.7	132.6	15.5	30.0	30.3	10.8	3.1
Year 2007											
Azumamugi	32.4	19.3	13.3	11.3	7.8	2.9	86.9	1.8	5.1	29.5	0.0
Kanto Nakate Gold	42.8	21.4	12.8	10.4	6.7	2.7	96.8	2.2	8.3	38.0	0.0
RIL, minimum	17.9	11.9	4.6	2.0	1.2	0.0	42.9	1.4	3.8	24.3	0.0
RIL, maximum	49.2	26.6	18.9	16.9	13.7	8.5	101.6	4.0	12.4	40.3	90.0
RIL, mean	35.6	19.6	12.6	9.7	5.9	2.8	83.6	2.3	7.1	32.1	7.7
RIL, SD	6.2	3.2	2.7	2.8	2.5	1.6	12.3	0.6	2.1	3.0	18.8
CV (%)	17.4	16.2	21.4	29.5	42.6	58.2	15.5	26.3	29.0	9.3	15.5

[a] *IN1* to *IN6* the first to sixth internode, *CUL* culm length, *SIL* spike internode length, *SPL* spike length, *TPN* triplet number, *LDG* lodging

[b] Lodging in year 2001 (upper) and 2002 (lower)

[c] Standard deviation

[d] Genotypic coefficient of variation

Table 2 Analysis of variance for the components of plant height

Source	df	IN1	IN2	IN3	IN4	IN5	IN6	CUL	SIL	SPL	TPN	LDG
Genotype	98	69.82**	17.17**	10.85**	12.34**	9.67**	3.57**	381.64**	0.83**	8.23**	15.86**	849.79*
Year	1	433.03**	1.30	8.65*	0.11	20.99**	170.00**	428.04**	0.12**	4.38**	171.06**	24772.10**
Error	98	12.23	2.97	1.71	1.73	1.43	0.55	43.28	0.02	0.25	3.88	539.48
Heritability (h^2b)		0.82	0.83	0.84	0.86	0.85	0.85	0.89	0.98	0.97	0.76	0.37

Analysis of variance of the randomized complete block design (a year as block) has been performed

*,** Significant at the 5 and 1% levels, respectively

Table 3 QTL for plant height components detected by composite interval mapping over two seasons

Trait[a]	Chr.	2005					2007					Locus
		Flanking markers	Pos. cM	LOD	AE[b]	PVE[c]	Flanking markers	Pos. cM	LOD	AE	PVE	
IN1	2HL	*vrs1*, *MWG503*	90.2	4.64	−1.63	0.056	ND[d]					*vrs1*
	3HL	*uzu*, *e06m30.10.1*	65.0	26.25	−5.02	0.516	*ABA001*, *uzu*	63.2	10.83	−5.82	0.393	*uzu1*
	5HL	ND					*e14m27.4.4*, *MWG2230*	105.2	3.62	3.54	0.104	*Eam5*
	7HL	*EBmag757*, *ABG608*	144.1	5.36	1.73	0.066	ND					*qCUL.ak-7H*
IN2	3HL	*uzu*, *e06m30.10.1*	65.0	14.79	−2.00	0.359	*ABA001*, *uzu*	63.2	5.21	−2.33	0.231	*uzu1*
	5HL	ND					*e14m27.4.4*, *MWG2230*	105.2	5.02	2.40	0.160	*Eam5*
	7HS	*cMWG704*, *e11m17.10.2*	89.0	5.66	−1.13	0.124	ND					*dsp1*
	7HL	*e12m22.10.2*, *EBmag757*	141.8	6.82	1.26	0.140	ND					*qCUL.ak-7H*
IN3	3HL	*uzu*, *e06m30.10.1*	65.0	8.43	−1.05	0.186	ND					*uzu1*
	7HS	*cMWG704*, *e11m17.10.2*	81.0	6.10	−0.82	0.126	*HVCMA*, *ABG701*	75.0	4.26	−1.30	0.138	*dsp1*
	7HL	*EBmag757*, *ABG608*	144.1	11.72	1.26	0.293	*EBmag757*, *ABG608*	142.1	5.62	1.48	0.174	*qCUL.ak-7H*
IN4	4HL	*EBmac0679*, *e12m19.10.2*	89.1	3.44	0.81	0.105	*EBmac679*, *e12m19.10.2*	89.1	3.31	1.09	0.108	*sgh1*
	5HL	*e07m25.3*, *Sgh2*	123.8	6.38	−0.97	0.143	ND					*Sgh2*
	7HS	*cMWG704*, *e11m17.10.2*	81.0	3.25	−0.67	0.073	*HVCMA*, *ABG701*	73.0	4.89	−1.29	0.151	*dsp1*
	7HL	*EBmag757*, *ABG608*	141.8	10.50	1.32	0.282	*EBmag757*, *ABG608*	141.8	4.38	1.18	0.116	*qCUL.ak-7H*
IN5	4HL	*e04m32.8*, *HVM67*	101.6	4.14	0.72	0.101	*e04m32.8*, *HVM67*	99.6	4.61	0.99	0.131	*sgh1*
	5HL	*MWG2230*, *e12m26.9*	114.3	10.34	−1.30	0.335	ND					*Sgh2*
IN6	2HS	EBmac684, e13m23.6	52.0	5.86	−0.45	0.128	e13m23.6, e13m31.7.1	52.6	3.69	−0.54	0.099	*Ppd-H1 (Eam1)*
	3HL	Bmag13, e06m30.8.3	118.1	3.79	0.34	0.076	ND					Unknown
	4HL	*e04m32.8*, *HVM67*	105.6	3.68	0.35	0.075	*e04m32.8*, *HVM67*	99.6	4.03	0.57	0.109	*sgh1*
	5HL	*MWG2230*, *e12m26.9*	116.3	7.38	−0.56	0.202	*Sgh2*, *e12m19.9.1*	127.5	4.26	−0.58	0.113	*Sgh2*
CUL	2HL	*MWG801*, *vrs1*	80.2	4.48	−3.55	0.068	ND					*vrs1*
	3HL	*uzu*, *e06m30.10.1*	65.0	19.21	−8.84	0.380	*uzu*, *e06m30.10.1*	64.9	19.71	−9.43	0.463	*uzu1*
	5HL	*e07m25.3*, *Sgh2*	123.8	2.97	−2.76	0.040	ND					*Sgh2*
	7HS	*cMWG704*, *e11m17.10.2*	87.0	3.89	−3.33	0.060	*cMWG704*, *e11m17.10.2*	87.0	5.56	−4.75	0.100	*dsp1*
	7HL	*EBmag757*, *ABG608*	141.1	12.86	6.51	0.226	*EBmag757*, *ABG608*	141.8	8.55	5.38	0.155	*qCUL.ak-7H*
SIL	2HL	*EBmag793*, *e11m19.3*	123.9	16.35	0.34	0.213	*EBmag793*, *e11m19.3*	123.9	5.98	0.30	0.163	*qSIL.ak-2H*
	3HL	*uzu*, *e06m30.10.1*	65.0	18.66	−0.37	0.247	*ABA001*, *uzu*	63.2	6.39	−0.33	0.200	*uzu1*
	7HS	*cMWG704*, *e11m17.10.2*	85.0	25.55	−0.45	0.397	*HVCMA*, *ABG701*	75.0	9.88	−0.36	0.258	*dsp1*
SPL	2HL	*e11m19.3*, *ABG613*	124.7	13.05	0.89	0.168	*EBmag793*, *e11m19.3*	123.9	8.43	1.16	0.221	*qSIL.ak-2H*
	3HL	*uzu*, *e06m30.10.1*	65.0	18.50	−1.12	0.268	*ABA001*, *uzu*	63.2	8.09	−1.16	0.219	*uzu1*
	7HS	*cMWG704*, *e11m17.10.2*	85.0	24.15	−1.31	0.394	*HVCMA*, *ABG701*	75.0	10.35	−1.21	0.252	*dsp1*
TPN	1HL	*ABC261*, *ABG055*	149.9	3.18	0.80	0.058	ND					*eam8 (eak)*
	2HS	*e13m23.6*, *e13m31.7.1*	52.6	14.74	−2.01	0.335	ND					*Ppd-H1 (Eam1)*
	2HL	ND					*vrs1*, *MWG503*	82.2	3.35	−2.16	0.115	*vrs1*
	3HL	ND					*e12m19.9.2*, *EBmac0541*	134.2	2.28	−1.84	0.082	Unknown
	5HL	*Sgh2*, *e12m19.9.1*	127.5	6.61	−1.14	0.117	ND					*Sgh2*
LDG[e]	1HS	*e12m19.4.1*, *Bmac0090*	40.2	9.04	17.57	0.266	ND					Unknown
	2HL	ND					*e15m19.8.1*, *MWG801*	73.81	3.72	6.66	0.123	*vrs1*
	3HS	ND					*e14m27.4.1*, *e15m19.7*	41.61	3.37	−5.97	0.098	Unknown
	7HL	*ABG608*, *e11m20.8*	142.1	4.91	13.57	0.131	*ABG608*, *e11m20.8*	141.8	3.07	6.15	0.103	*qCUL.ak-7H*

[a] *IN1* to *IN6* first to sixth internode, *CUL* culm length, *SIL* spike internode length, *SPL* spike length, *TPN* triplet number, *LDG* lodging

[b] Additive effect of 'Azumamugi' allele

[c] Proportion of phenotypic variance explained by the detected QTLs

[d] Not detected

[e] Data of years 2001 (left) and 2002 (right)

Discussion

The *qCUL* locus has a significant effect on the IN1–IN4 (Fig. 3). Its effect on IN3 and IN4 was greater than that of either *uzu1* or *dsp1*. Genes (QTL) affecting plant height in barley have been detected on the short arm of chromosome 7H in a number of previous studies (Hayes et al. 1993; Zhu et al. 1999; Marquez-Cedillo et al. 2001), but the position

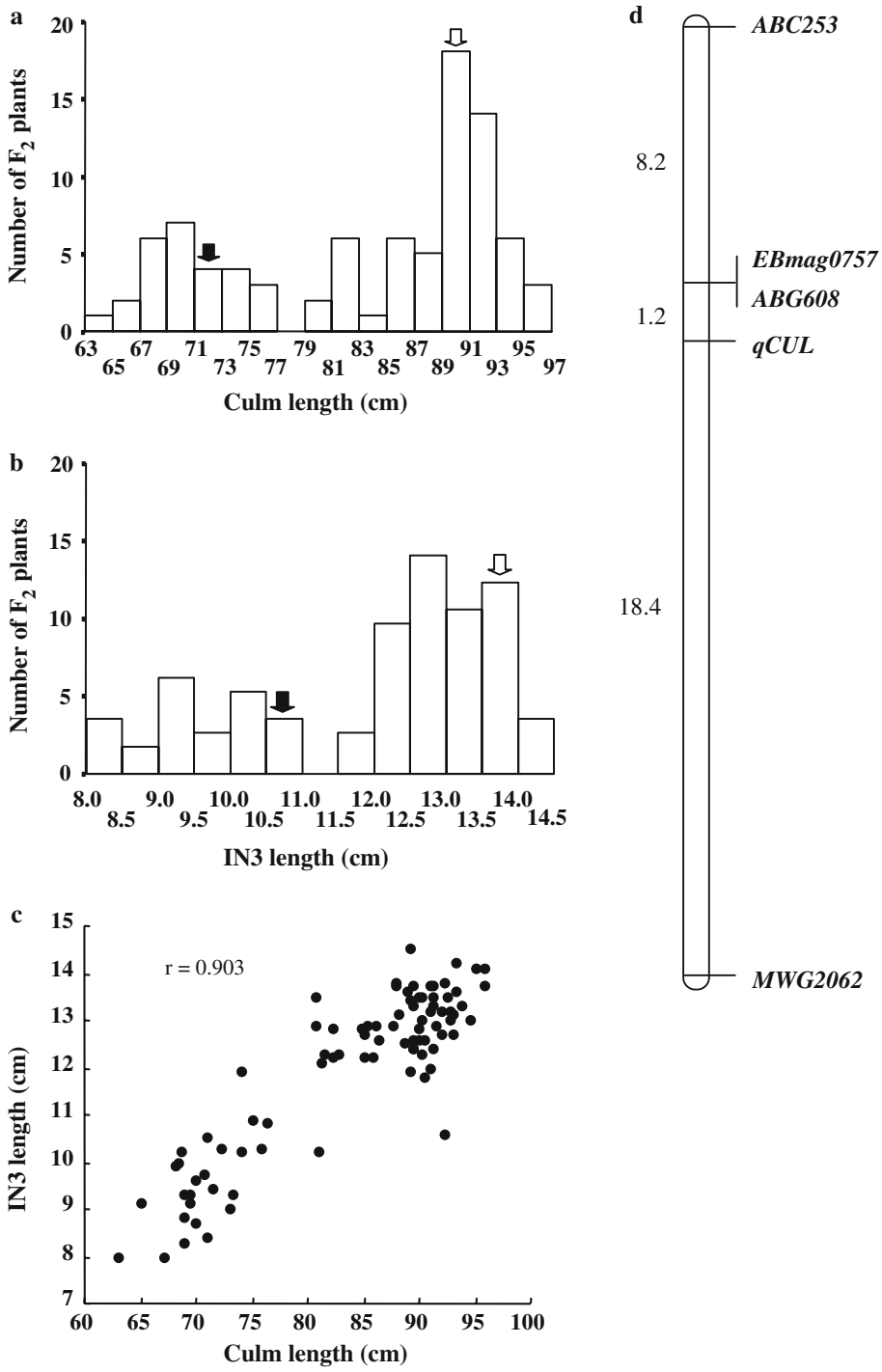


Fig. 2 *qCUL* segregates as a single Mendelian gene on the long arm of chromosome 7H. Frequency distribution of F_2 plants derived from the cross of NIL#11 (*solid arrow*) × AZ (*open arrow*) for CUL (**a**) and IN3 (**b**) and their correlation (**c**). The linkage map of *qCUL* from an F_2 population bred from the cross NIL#11 × AZ (**d**)

of these QTL close to *ABG701* (Kleinhofs 2002) suggests that they reflect the pleiotropic action of *dsp1*, rather than representing determinants of exclusively plant height. However, the parental lines were not known to carry *dsp1* gene. Therefore, we preserve a possibility that these effects are associated with unknown factors. A plant height QTL close to *Bmag0120* on the long arm of chromosome 7H was responsible for a significant proportion of the phenotypic variance in a population analyzed by Pillen et al. (2003). Laurie et al. (1995) identified the *eps7L* locus on chromosome 7HL for heading time, but we did not detect this QTL in our mapping population (Sameri and Komatsuda 2004). *Bmag0120* maps 15 cM proximal to *qCUL*, and the confidence interval for *qCUL* did not extend to *Bmag0120*. Therefore, Pillen et al. (2003) are unlikely to have studied the same QTL for plant height.

Minimizing the elongation of the lower internodes is an important strategy for increasing lodging tolerance

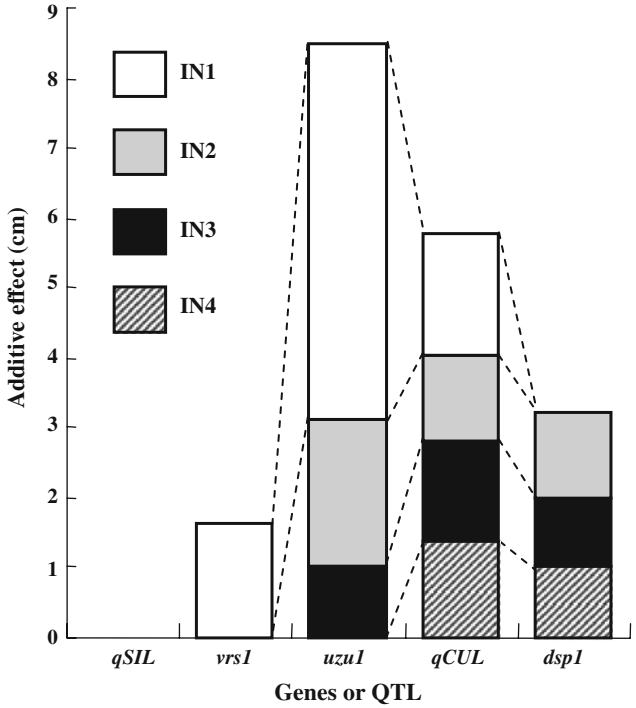


Fig. 3 Additive effects of *qSIL.ak-2H, vrs1, uzu1, qCUL (qCUL.ak-7H)* and *dsp1* on each internode length. The data were obtained from average additive effects of major genes in 2 years (2005 and 2007). The KNG allele at *qCUL* shortens culm length

(Yamamoto et al. 2001). Deepwater rice revealed floating ability and their lowest internodes can elongate in response to rises in water level (Hattori et al. 2008; Kawano et al. 2008). This is an adaptation to environmental change, where the importance of the lower internodes for the control of plant height was discovered. In this study, *qCUL* has a major effect on IN3 and IN4, it also has a significant effect on lodging tolerance. The *qCUL* allele of taller parent (KNG) decreased the culm length and much more favorable than reduction by other dwarf or semi-dwarf gene. KNG is a Japanese two-rowed cultivar with a spring habit bred using the following pedigree, Golden Melon/Shikoku//Kinki Shu. Kinki Shu is a selection from Golden Melon and Shikoku is Japanese landrace. Theoretically, in KNG, the proportion of Shikoku is 25%, while that of Golden Melon is 75%. However, KNG is morphologically and physiologically much more similar to Golden Melon than to Shikoku, presumably due to selection in the breeding process. KNG is carrying the oldest known source of a semi-dwarf gene, which is associated with this region 7HL. The economic value of this gene (*qCUL*) can be reflected by its likely presence in Japanese cultivars, Chinese cultivars (Zhang and Zhang 2003), ICARDA barleys from the CIMMYT program and some Australian cultivars. This point is yet to be proven, but the information in this paper would help considerably in planning experiments.

denso gene, located on chromosome 3HL (Bezant et al. 1996; Dahleen et al. 2005), is separated from *uzu1* by some 40 cM (Sameri et al. 2006). The *denso* gene is associated not solely with plant height, but also with heading date and some yield components (Laurie et al. 1995; Yin et al. 1999). Semi-dwarf cultivars are common in Japan and Korea (Dahleen et al. 2005), but unlike in European germplasm, culm length is limited by the presence of *uzu1*. In AZ, the *qCUL* allele increased culm length, and thus compensated in part for the height reduction imposed by the simultaneous presence of *uzu1* and *dsp1*. Semi-dwarf barleys tend to have rather brittle culms (Madic et al. 2006), which increase their susceptibility to lodging.

The QTLs controlling TPN at chromosomes 1HL and 2HS might correspond to *eam8* and *Ppd-H1*, respectively. The *eam8* naturally occurred in a few Japanese two-rowed barleys for example Kinai 5 and Kagoshima Gold (Franckowiak et al. 1997). It remains unclear whether the QTL (at the interval *ABC261–ABG055)* and *eam8* are identical, but AZ allele exerts a moderate effect to later heading date. *Ppd-H1* can be expressed even partially in our trials because day length of trial changed from 11 h 22 min (13 October for sowing), 9 h 43 min (midwinter) and 14 h 37 min (20 June for maturation). Position of *lin1* (lesser internode number 1) gene was located on chromosome 2HL and 17.1 cM proximal from *vrs1* (Franckowiak 2002), therefore the *lin1* is not the QTL.

There are several studies on plant height genes for example the semidwarf 1 (*sd-1*) gene in rice and reduced height 1 (*Rht1*) gene in wheat, which have played similar roles in height reduction associated with significant yield increases (Sasaki et al. 2002; Peng et al. 1999). The *uzu* gene of barley also shows a semidwarf plant type whose height is reduced to 80–90% of its normal counterpart under normal cultivation (Chono et al. 2003; Saisho et al. 2004). However, the reason why some genes only influence some internodes has not been clarified. Short-statured cultivars have been developed by cereal breeders worldwide to reduce lodging and increase grain yield (Hellewell et al. 2000). Semi-dwarf barley has not gained the same level of acceptance as have semi-dwarf wheat and rice, probably because *denso* in Europe (*sdw1* in north America), and *uzu1* and *dsp1* in Japan and Korea produce pleiotropic effects on other agronomic traits such as yield and yield components (Sameri et al. 2006; Sameri and Komatsuda 2007). *qCUL*, by contrast, does not appear to suffer from this disadvantage (Sameri et al. 2006 and Sameri and Komatsuda 2007), and thus should be a useful resource for the breeding of short-stature barleys. Although its effect on height should be rather small, but pyramiding or combining other favorable genes which have not pleiotrophic to other traits except height is a key for lodging resistance. While the majority of QTL discovery

programs rely on segregation patterns in mapping populations, near-isogenic lines (NILs) represent a particularly effective resource for identifying and characterizing the effect of individual QTL, because the genetic background is constant across all test individuals, and thus does not interfere with the estimates of genetic location and size of the QTL effect (Yamamoto et al. 2001). NILs have been applied for gene discovery in barley (Pillen et al. 2003; Li et al. 2005), and beyond this for the cloning of several plant QTL (Frary et al. 2000; Yano et al. 2000; Ashikari et al. 2005). The flanking markers *ABG608* and *EBmag0757* can be readily exploited for marker-assisted selection of *qCUL*. Given that the estimated equivalence between physical and genetic distance in the *qCUL* region is ~500 kb/cM (Kunzel et al. 2000), the tight linkage with the STS locus *ABG608* implies that map-based cloning of the gene may perhaps be feasible. For this purpose, it is necessary to improve the high resolution map by increasing the number of markers in both side of the QTL.

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